were used to observe the dynamics of right- to left-handed DNA transitions under tension. We found that this transition is highly hydrogen bond dependent. Although DAP and normal DNA exhibit similar patterns of conformational change, DAP DNA converted to left-handed DNA at lower tension. Also, we found pronounced hysteresis for normal DNA upon re-winding from a left- to right-handed form, which indicated the that heterogeneity of the number of hydrogen bonds between base pairs in normal DNA contributes to non-equilibrium conformational changes. The results suggest that hydrogen bonding can significantly affect cellular processes requiring certain under-wound DNA conformations, and DAP DNA provides a sequence-independent model for studying the effects of hydrogen bonding on conformational changes of DNA.

1394-Pos Board B164

The Mechanical Properties of Double-Stranded RNA in Response to Force and Torque

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While the response of double-stranded DNA to applied forces and torques has been measured with exquisite precision, much less is known about double-stranded RNA (dsRNA). We have recently developed a ''polymerase-stall'' labeling method that allows us to generate fully double-stranded RNA constructs carrying multiple biotin and digoxygenin labels at opposite ends. Using the functionalized dsRNA constructs in a range of a complementary magnetic tweezers assays, we have probed the elastic properties of dsRNA and, in addition, determined force and torque induced structural transitions that go beyond linear response behavior. Using conventional magnetic tweezers, we have determined the bending persistence length and the stretch (or Young's) modulus of dsRNA. Employing our novel magnetic torque tweezers[1] and freely-orbiting tweezers [2] assays, we have probed the torsional persistence length of dsRNA and its twist-stretch coupling. While the elastic properties are, overall, similar to dsDNA, we have discovered a striking difference in the energy landscape at the buckling torque under positive twist. These measurements of the fundamental properties of dsRNA can inform quantitative models of RNA function in vivo, for example of the packing of viral RNA genomes or of the mechanical function of double-stranded regions in functional RNAs.

[1] Lipfert, Kerssemakers, Jager & Dekker, Nature Methods (2010).
[2] Lipfert, Wiggin, Kerssemakers, Pedaci & Dekker, Nature Communications (2011).

1395-Pos Board B165

Single-Molecule Thermodynamics of Three Distinct DNA Structural Transitions under Large Tension

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Torsion unconstrained double-stranded DNA can undergo three structural transitions under large tension. At forces around 65 pN, a well known DNA overstretching transition occurs during which the DNA elongates by about 1.7-fold. Recent experiments have revealed two distinct structural transitions during DNA overstretching: a ''strand-unpeeling'' of one strand from the other, and a ''B-to-S'' transition to a mysterious double-stranded ''S-DNA''. In addition to DNA overstretching transition, DNA without nicks or free ends can also undergo an internal melting transition to two parallel DNA strands under tension at $>$ 100 pN in physiological solution conditions or under lower tension at sufficiently low salt concentration or high temperature. Here, we report thermodynamics measurements for the entropy and enthalpy changes during these transitions. In addition, the force-responses of these DNA structures are directly determined in Single-molecule experiments. With these experimentally determined thermodynamic and mechanical quantities, the selections of the transitions are summarized in phase diagrams.

1396-Pos Board B166

Bandpass Filtering of DNA Elastic Modes using Confinement and Tension

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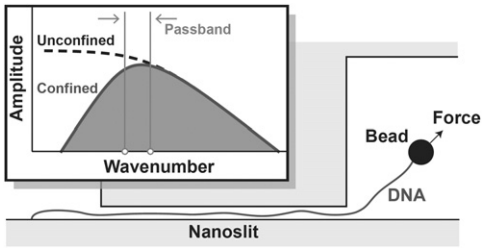
During a variety of biological and technological processes, biopolymers are simultaneously subject to both confinement and external forces. While significant efforts have gone into understanding the physics of polymers that are only confined, or only under tension, little work has been done to explore the effects of the interplay of force and confinement. Here, we study the combined effects of stretching and confinement on a polymer's configurational freedom. We measure the elastic response of long double-stranded DNA molecules that are partially confined to thin, nanofabricated slits. We account for the data through a model in which the DNA's short wavelength transverse elastic modes are cut off by applied force and the DNA's bending stiffness, while long-wavelength modes are cut off by confinement. Thus, we show that confinement and stretching combine to permit tunable bandpass filtering of the elastic modes of long polymers.



1397-Pos Board B167

Testing Models For DNA Elasticity on Short Length Scales by Simulating DNA Supercoiling under Tension

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The worm-like-chain (WLC) model is widely used to describe the energetics of DNA bending. However, for length scales much shorter than the persistence length the validity of a purely harmonic bending potential has been questioned by recent experiments. So-called sub-elastic chain (SEC) models were proposed that predict a lower elastic energy of highly bent DNA conformations. Until now, no unambiguous verification of these models has been obtained since probing the elasticity of DNA on short length scales remains challenging. Here we address this issue by modeling single molecule supercoiling experiments of DNA under tension using coarse-grained Monte Carlo simulations. Twisting of stretched DNA is accompanied by an abrupt decrease of the DNA extension at a critical supercoil density due to buckling of the molecule. This transition is caused by an energetic offset due to the strongly bent end-loop of the forming superhelical structure. While simulations based on WLC bending energetics could quantitatively reproduce the buckling measured in magnetic tweezers experiments, the buckling almost disappears for the tested linear SEC model. Thus, our data support the validity of a harmonic bending potential even for strongly bent DNA down to bending radii of 3.5 nm.

1398-Pos Board B168

Calculation of the Energy of a Bio-Polymer as a Function of End-To-End Extension

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When considering the folding of a biopolymer (DNA, RNA or protein) it is natural to express the energy of the system as a function of conformation. However, in single molecule manipulation experiments the system is most naturally characterized in terms of the end-to-end distance of the polymer, and the energy landscape itself can be measured as a function of the end-to-end distance. The relationship between energy as a function of conformation and energy as a function of end-to-end distance is extremely complex. We introduce a physical model which allows the energy landscape as a function of end-to-end distance to be calculated from knowledge of the energy as a function of conformation of the polymer. Using optical trap based experiments we show that the measured energy landscape of a DNA stem-loop structure agrees with the landscape calculated from our model with extremely high precision. We believe the combination of our model with high resolution optical trap techniques will facilitate direct measurement of the hybridization energy of nucleic acid base pairs and sequencing of double stranded RNA and DNA.

1399-Pos Board B169

Nonlinear Elasticity of DNA Bending

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Double stranded (ds) DNA has been used as a ''molecular spring'' to mechanically control the conformation of proteins, ribozymes and peptides [1]. It is thus interesting to characterize quantitatively the mechanics of DNA springs in the relevant regime of sharp bending ($x<<2L<<l_d$, where x is the end-to-end distance (EED), $2L$ the contour length of the DNA, l_d ~50 nm the persistence length). This nonlinear elasticity regime is also relevant to several cell biology mechanisms, such as DNA packaging and transcription regulation.
We address the problem with the stressed molecules consisting of a bent ds DNA part and a stretched ss part: essentially a system of two coupled nonlinear springs. We measured the elastic energy of these molecules with two different equilibrium methods, based on dimer formation [2, 3] and melting curve

analysis [4] respectively. We deduce the bending energy of the ds part as a function of EED x. The finding is that the DNA kinks at a critical bending torque τ_c ~30 pN x nm, entering a nonlinear regime where the maximum torque is constant (= τ_c). We derive an analytic expression for the bending energy valid in the linear and nonlinear regimes [3].The critical torque τ_c introduces a universal energy scale ~12 kT in the physics of DNA bending.

[1] G. Zocchi, ''Controlling proteins through molecular springs'', *Ann. Rev. Biophys.* 38, 75-88 (2009).
[2] H. Qu, C.-Y. Tseng, Y. Wang, A. J. Levine, and G. Zocchi, ''The elastic energy of sharply bent nicked DNA'', *Europhys. Lett.* 90, 18003 (2010).
[3] H. Qu and G. Zocchi, ''The complete bending energy function for nicked DNA'', *Europhys. Lett.* 94, 18003 (2011).
[4] H. Qu, Y. Wang, C.-Y. Tseng, and G. Zocchi, ''Critical torque for kink formation in double stranded DNA'', accepted by *Phys. Rev. X* (2011).

1400-Pos Board B170
Simulation of Plectonemic Supercoil Diffusion Along Extended DNA by Brownian Dynamics
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DNA supercoils arise from processes such as replication, transcription and the nearly 10000-fold compaction of long DNA molecules into the cell nucleus. Because of their potential to enhance or repress cellular processes (e.g. replication, transcription, and recombination), supercoils must be carefully regulated. In fact, chemotherapy drugs have been designed to impede topoisomerase enzymes that are responsible for the regulation of supercoils. Motivated by the biological significance of supercoils, several experimental and computational studies have focused on probing their structure and dynamics. For example, recent single molecule experiments have uncovered a dynamic buckling transition while interrogating the torsion-extension relationship for supercoils. As another example, Brownian Dynamics (BD) simulations of kilo base-pair lengths of circular DNA have explored the sensitivity of site juxtaposition times to supercoiling. Unfortunately, however, there remain many fundamental questions concerning the structure and dynamics of supercoils. In this study, our objective is to quantify the diffusion constant of plectonemic supercoils along stretched DNA. To this end, we employ BD simulations of kilo base-pair lengths of DNA with biologically relevant levels of supercoiling and extension. From these simulations, we quantify the diffusion constant for plectonemes and further compute site juxtaposition and plectoneme nucleation timescales.

1401-Pos Board B171
A Single Molecule Kinetic and Thermodynamic Approach to Oligonucleotide Duplex Formation
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The formation of the canonical double stranded helix of DNA and RNA, through oligonucleotide hybridization, is a ubiquitous process in biology. Here we describe a single molecule fluorescence resonance energy transfer (smFRET) study that probes the formation and dissociation of a short (6-9 base pairs) double stranded DNA duplex. Kinetic measurements on this construct reveal that rates of duplex formation are not diffusion limited. In fact, the rate constants of formation are 2-3 orders of magnitude slower than estimated with a simple diffusion model. Subsequently, the duplex formation/dissociation (kon and koff) rates were measured as a function of Na+ concentration and both were found to change in a sigmoidal fashion with [Na+]. The measured values of koff decreased by a factor of four (0.7(2) s-1 to 0.14(5) s-1) between 25 mM and 1 M Na+. Surprisingly, kon was found to be much more sensitive to the [Na+], increasing by more than a factor of 40 (0.6(6) s-1 to 4.4(6) s-1) over the same range. These changes combine give a >100 fold change in the unimolecular equilibrium constant (Keq). Finally, the temperature dependence of Keq was used to dissect the free energy of duplex formation (ΔG°) into its enthalpic (ΔH°) and entropic (ΔS°) components. While ΔH° remains negative under all conditions, increasing the [Na+] reduced the magnitudes of both ΔH° and ΔS°. Given that ΔG° decreases with increasing [Na+], the magnitude of ΔS° must decrease faster than the unfavorable reduction in the magnitude of ΔH°. These insights highlight the dynamic nature of DNA duplexes on the time scale (seconds to minutes) of many cellular processes. Additionally, these data may aid in developing and evaluating new methods for modeling the thermodynamics of DNA duplex formation.

1402-Pos Board B172
Measuring Sequence-Dependent DNA Looping Kinetics
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Looping of double-stranded DNA occurs in many important biological processes. The best illustrated example of this is nucleosome formation where DNA loops almost twice around the histone octamer core. At short length scales around or below ~200-bp, looping efficiency of DNA is known to depend strongly on its sequence, but the physical basis of this dependence is not completely understood. Although looping equilibrium of double-stranded DNA has been extensively studied by the ligation-based cyclization assay, experimental studies on its looping kinetics are rare. Thus, to study how DNA sequence affects its looping kinetics, we developed an assay based on single-molecule Fluorescence Resonance Energy Transfer (FRET), which allows us to observe reversible looping of ~190-bp long double-stranded DNA in real time. Initially, we measured looping kinetics of two different DNA molecules that are known to exhibit opposite propensities to form a nucleosome *in vitro*, referred to as the nucleosome DNA (high nucleosome occupancy) and the anti-nucleosome DNA (low nucleosome occupancy). Under all salt conditions tested, the nucleosome DNA was observed to loop faster than the anti-nucleosome DNA. Surprisingly, the looping rate of anti-nucleosome DNA decreased with increasing salt concentration in contrast to the nucleosome DNA. Our findings support a model where looping kinetics of double-stranded DNA is accelerated by formation of local defects in the secondary structure. We are further testing a series of DNA molecules that vary in nucleosome preference to study the correlation between physical properties of DNA and the chromatin code.

1403-Pos Board B173
Visualizing Higher-Order Conformations in Single DNA Molecules
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Not simply a linear molecule, DNA is found in many higher-order conformations such as loops, plectonemes and condensed regions. Some of these conformations are mediated by proteins and others occur naturally. Much mechanical work has been done investigating these structures in single DNA molecules but, to date, none of these structures have been imaged in single-molecule experiments. Here, we present data visualizing some of these structures using combined fluorescence and force measurements and compare our data with previous mechanical measurements.

1404-Pos Board B174
Hidden Complexity in the Isomerization Dynamics of Holliday Junctions
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Persistent heterogeneity has recently been identified in single molecule measurements of enzymes and ribozymes, but its systematic quantification and structural origin remain elusive. Here, we addressed these issues by analyzing isomerization dynamics of Holliday Junctions (HJs) as a test case. Even for HJs that retain relatively simple architectures, the molecule-to-molecule variation in dynamics persists over long observation time (Tobs), implying ergodicity breaking of HJ conformational space. An annealing experiment using Mg2+ pulse, which enables interconversion between HJ trajectories with different patterns, and the architecture of HJ suggest that the persistent heterogeneity of HJs reflects various internal multiloop topologies stabilized by Mg2+ ions.

1405-Pos Board B175
Concentration-Dependent Structural Transitions of Human Telomeric DNA Sequences
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Oligodeoxyribonucleotides (ODNs) composed of four repeats of the human telomeric sequence d(TTAGGG) can assume several conformations depending on the cation species present, the presence of additional bases at the 5' or 3' end, and on the sample preparation. The secondary structure of these molecules is stabilized by intramolecular G-quartets. Structural studies have shown that H-Tel, [d(5'-AGGG(TTAGGG)$_3$-3')], an ODN composed of repeats of the human telomeric sequence exhibits significantly different conformations in the presence of different cations. We have investigated the effect of the concentration of the oligonucleotide on the conformation and self-association of H-Tel and two ODNs that differ from H-Tel only in the terminal bases, Hybrid-1, d(5'-AAAGGG(TTAGGG)$_3$AA-3'), and Hybrid-2, d(5'-TTAGGG(TTAGGG)$_3$TT-3'). The equilibrium structures formed by H-Tel were studied using circular dichroism spectroscopy (CD), Raman spectroscopy, dynamic light scattering (DLS), and analytical ultracentrifugation. We observed that in aqueous solutions containing 100 mM or greater NaCl, at 2 mM (strands) folded monomers of H-Tel self-associate to form species consisting of at least four monomers, i.e. (H-Tel)$_4$. The formation of the associated species was reversible; monomers formed rapidly upon dilution to 20 μM (strands). We were able to observe the time-dependence of the association using CD and DLS,